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ELEANOR ROOSEVELT INSTITUTE FOR CANCER RESEARCH, INC.
FLORENCE R. SABIN LABORATORIES FOR GENETIC AND DEVELOPMENTAL MEDICINE

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August 11, 1981

Dr. Robert C. Hockett
Research Director
The Council for Tobacco Research
- U.S.A., Inc.
110 East 59th Street
New York, New York 10022

Dear Dr. Hockett:

I am writing for assistance in the conduct of a research program in this laboratory dealing with the nature of malignancy, in general, and small cell cancer of the lung, in particular.

The program which we have designed depends upon scientific developments originating in our own laboratory, plus a new finding which has just been established in the laboratory of Dr. Minna at the National Cancer Institute. Together, these observations open up important new possibilities that promise fundamental illumination of lung cancer.

We have established the following facts:

1) Administration of cyclic AMP derivatives coordinately removes all of the transformation stigmata of the transformed malignant cell CHO-K1.

2) The restoration of the normal cell properties by cyclic AMP proceeds through the intermediary agency of the re-establishment of an organized cytoskeletal system which had been disorganized in the transformed cell.

3) Experiment has demonstrated that the cytoskeleton is necessary for the normal protein biosynthesis associated with the ability to exercise reproductive control, and that the normal cytoskeleton is also required for control of the constancy of chromosomal constitution in dividing cells.

4) It was demonstrated that cyclic AMP will also reverse all of the five, critical transformation characteristics of normal vole fibroblasts transformed by avian sarcoma virus containing the src gene, and that both cyclic AMP and the src gene product affect the same metabolic pathways. These experiments demonstrate that, in at least these two different transformed cell systems, an antagonism between cyclic-AMP mediated phosphorylation, which affects serine and threonine moieties, as opposed to tyrosine phosphorylation by the src gene product appears to determine whether a cell behaves as a normal or transformed entity.

5) Other experiments carried out in this laboratory have demonstrated that the procedures of recombinant DNA can be combined with somatic cell genetic techniques, so as to make possible regional mapping of DNA segments without the necessity for isolation of specific messenger RNA's. The principle of the method requires the following steps: preparation of hybrids containing a single human chromosome along with the standard Chinese hamster ovary cell chromosome; quantitative isolation of the human DNA from such cells so as to form a library from a single human chromosome; preparation of a series of deletion mutants in which successive hybrids are prepared with different terminal deletions from each end of the human chromosome in question; use of the Southern Blotting Technique to determine whether standard DNA probes, prepared in the second step of this procedure, attach or fail to attach to the DNA from the panel of standard hybrid deletion cultures, so that the mapping position of each DNA probe can be determined; and use of two-dimensional electrophoresis-electrofocusing making it possible to identify human peptides associated with each of these mapping regions, and to study regulation of the expression of genes involved.

6) A recent development in Dr. Minna's laboratory has established that small cell cancer of the lung, which includes oat cell carcinoma, is associated with a deletion in human chromosome 3. This observation now makes possible application of the approaches described in the preceding paragraphs to this extremely common form of human cancer.

Samples of these cancers will be obtained directly from Dr. Minna and from patients in Colorado General or other hospitals. Cultures will be established and will be studied with respect to the following procedures:

A) The mapping position of the deletion associated with lung cancer will be established with the highest possible resolving power.

B) Hybrids will be established with human chromosome 3 and a series of terminal deletion mutants will be prepared; the human peptides whose expression is altered by the presence of the specific deletion associated with small cell lung cancer will be identified by two-dimensional electrophoresis-electrofocusing; study will be undertaken

to identify these peptides chemically and to determine their sequence by means of the sequentor devised by Dr. Leroy Hood, of Cal-Tech, who has offered to collaborate in these experiments; and finally, the action of cyclic AMP derivatives and of reagents which disorganize the cytoskeleton will be studied on the expression of these particular peptides in malignant and normal lung cells. The change in patterns of phosphorylation brought about in such cells by cyclic AMP derivatives and other agents which affect cytoskeletal organization will also be studied.

These studies promise to elucidate the effects of cyclic AMP and specific kinds of phosphorylation on the genesis and perhaps the reversal of lung cancer manifestations and may lead to the possibility of identification of specific peptides which may be involved in these processes.

Sincerely,

Theodore T. Puck

Theodore T. Puck, Ph.D.
Director, Institute for
Cancer Research
Professor of Biochemistry,
Biophysics and Genetics

TTP/kc
cc: Dr. Frederick Seitz
P.S.) I will be glad to submit a budget and any other information you may require.